Miltenyi Biotec

REAfinity™ Antibodies

More specific. More impact.

Recombinant engineered antibodies display superior specificity and affinity, and therefore simplify flow cytometry analysis.

Introduction

Flow cytometry allows the sensitive detection of even very rare cell types based on specific marker antigens. However, the reliability of cell identification and accuracy of enumeration critically depend on the specificity of the marker antibody used for staining the cells. Miltenyi Biotec has developed REAfinity™ Antibodies, for a multitude of different human, mouse, and rat antigens.

Recombinant antibody engineering

In order to make the best antibodies in terms of antigen recognition and binding affinity, a premium antibody clone selection technology has been applied. Via this process the best clone for each specificity was established. Recombinant engineering of the respective antibody clones further enhanced their properties. The resulting REA clones are superior to the initial mouse or rat monoclonal antibodies.

Besides outstanding antigen binding characteristics, REAfinity Antibodies have various compelling benefits, which simplify and improve flow cytometry analysis:

- REA clones do not bind to Fcγ receptors. Therefore, no Fc receptor blocking step is required prior to cell labeling, which saves time and money.
- All REA clones have specifically mutated human IgG1 parts for Fc regions. Therefore, one universal isotype control per fluorochrome is sufficient, saving money and effort.
- REA clones contain only a single type of Ig heavy and light chain, resulting in high purity and lot-to-lot consistency. Ultimately, this leads to higher experimental reproducibility.
- Approximately 200 REA clones conjugated to FITC, PE, APC, VioBlue®, VioGreen™, PE-Vio770™, APC-Vio770™, PerCP-Vio700™, or biotin provide a high flexibility in multicolor applications.

REAfinity Antibodies are highly specific and allow for a more accurate cell analysis than mouse monoclonal antibodies

Compared to mouse monoclonal antibodies, binding of REA clones to target antigens results in less background signals (figs. 1–3). This is reflected by a higher resolution and easier distinction of target populations. Ultimately, this provides for a more accurate and quicker flow cytometry data analysis.

The process for the conjugation of fluorochromes to REA clones has been greatly improved, leading to optimal labeling efficiencies, i.e., degree of labeling (DOL) and fluorochrome/protein ratio. Combined with the low background binding of the REA clones, the results are higher mean fluorescence intensities (MFI) and stain indices (SI) (figs. 1–3).

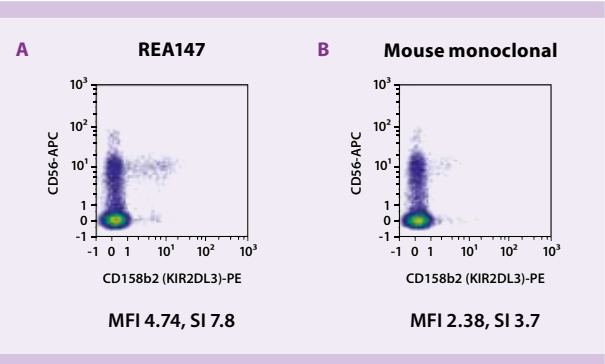

Figure 1: Specific detection of human CD158b2⁺ cells with REA147. PBMCs were stained with either a PE-conjugated REAfinity Antibody (A) or a PE-conjugated mouse monoclonal antibody (B) recognizing CD158b2. Cells were also stained with CD56-APC (# 130-100-698) and analyzed by flow cytometry on the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide (PI) fluorescence.

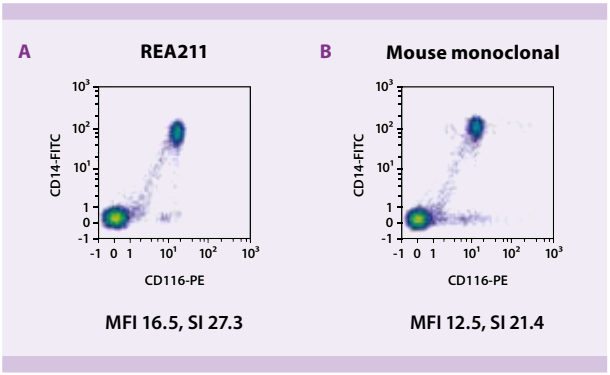

Figure 2: Specific detection of human CD116⁺ cells with REA 211. PBMCs were stained with either a PE-conjugated REAfinity Antibody (A) or a PE-conjugated mouse monoclonal antibody (B) recognizing CD116. Cells were also stained with CD14-FITC (# 130-080-701) and analyzed by flow cytometry on the MACSQuant Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide (PI) fluorescence.

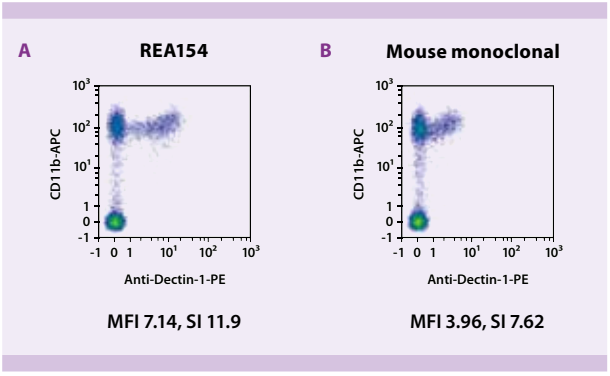

Figure 3: Specific detection of mouse Dectin-1⁺ cells with REA154. Bone marrow cells from BALB/c mice were stained with either a PE-conjugated REAfinity Antibody (A) or a PE-conjugated mouse monoclonal antibody (B) recognizing Dectin-1. Cells were also stained with CD11b-APC (# 130-102-493) and analyzed by flow cytometry on the MACSQuant Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide (PI) fluorescence.

REAfinity Antibodies show virtually no binding to Fcγ receptors

All REA clones are specifically engineered by introducing mutations in the human IgG1 Fc region. As a result, REA clones show virtually no binding to Fcγ receptors (fig. 4), and staining with REA clones eliminates the requirement for FcR blocking.

REAfinity Antibodies show only minimal background signals

In the absence of an FcR blocking reagent, staining with the mouse monoclonal antibody results in strong non-specific background signals (fig. 5A). Therefore, it is necessary to include an FcR blocking step prior to staining with the mouse monoclonal antibody (fig. 5B). In contrast, staining with the REA clone allows the specific detection of the CD158a⁺ target population, even without FcR blocking (fig. 5C).

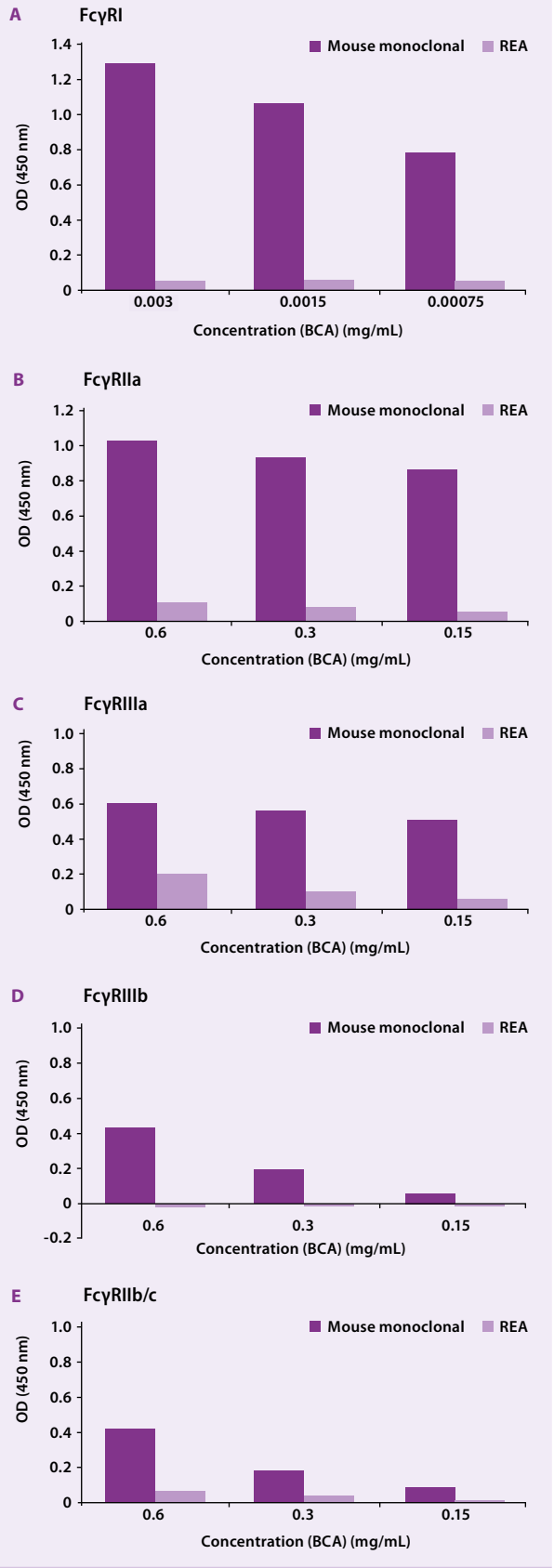

Figure 4: Binding of a mouse monoclonal antibody and a REAfinity Antibody to Fcγ receptors. Binding of a CD144-specific REAfinity Antibody and a mouse monoclonal antibody to the five cellular Fcγ receptors was compared using enzyme-linked immunosorbent assays. Binding of immobilized Fcγ receptors to a REA clone and mouse monoclonal antibody was detected using secondary antibodies conjugated to HRP. BCA: bicinchoninic acid.

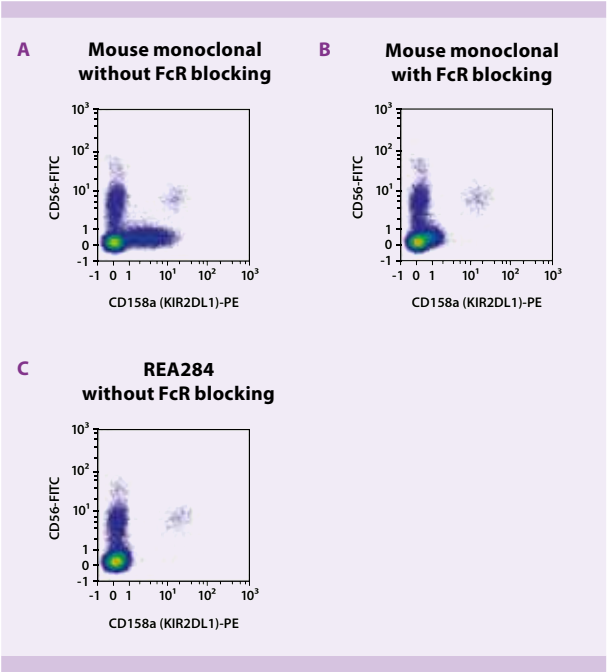

Figure 5: Staining of cells with a mouse monoclonal antibody or a REAfinity Antibody in the absence or presence of FcR blocking reagent. PBMCs were stained with either a PE-conjugated mouse monoclonal antibody (A, B) or a PE-conjugated REAfinity Antibody (C) recognizing CD158a. Cells were also stained with CD56-FITC (# 130-100-746) and analyzed by flow cytometry on the MACSQuant Analyzer. Staining with the mouse monoclonal antibody was performed either without (A) or with (B) pre-treatment with FcR blocking reagent. No FcR blocking reagent was included prior to staining with the REAfinity Antibody (C). Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide (PI) fluorescence.

Only one isotype control is required for flow cytometry analysis with all REA clones

Although REA clones show virtually no binding to Fcγ receptors, it is recommended to include a control (clone REA293) for monitoring non-Fc receptor–mediated binding of REA clones to cells. Non-specific interactions of the fluorochrome with the cell surface can be checked with fluorochrome-conjugated versions of clone REA293.

As all REA clones contain specifically mutated human IgG1 parts for constant regions, only one type of isotype control is required, namely the REA Control antibodies, which are based on clone REA293. This control antibody is available both for surface antigens and intracellularly expressed antigens.

REAfinity Antibodies contain only one type of heavy and light chain

In samples of mouse monoclonal antibodies very often mixtures of heavy and light Ig chains can be observed (fig. 6A). Even though these differences may not always become apparent, they lead to lot-to-lot variations and may also impact the reproducibility of experiments. As all REA clones are manufactured in mammalian cells and produced under highly controlled manufacturing processes, REAfinity Antibodies contain only one type of heavy and light chain (fig. 6B). Compared to the average mouse monoclonal antibody, REA clones are purer, have a higher lot-to-lot consistency and therefore are the reagent of choice for long-term studies.

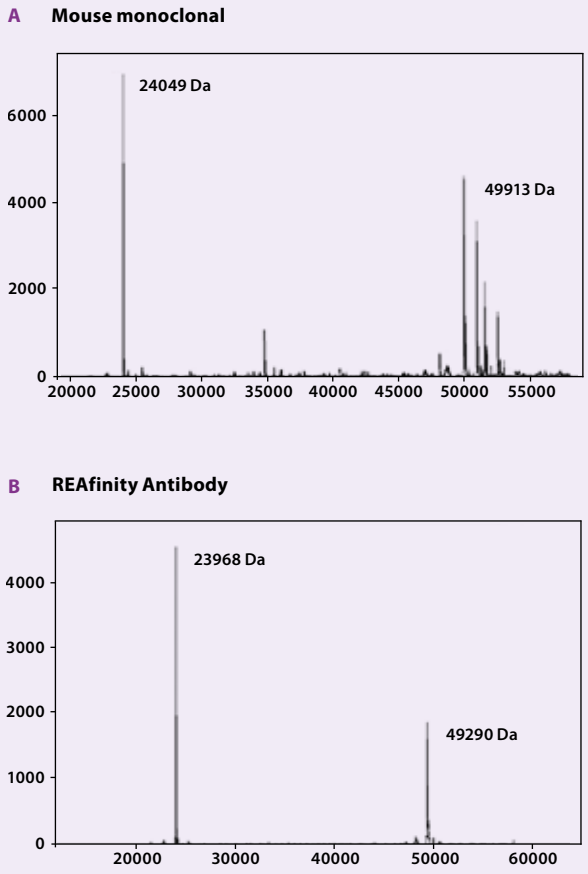

Figure 6: Mass spectrometry analysis of a mouse monoclonal antibody and a REAfinity Antibody. A mixture of Ig heavy and light chains can be observed in the mouse monoclonal antibody sample (A), whereas the REA clone (B) shows only one heavy chain (~50 kDa) and one light chain (~24 kDa).

Details on the REAfinity Antibody portfolio and answers to frequently asked questions can be found at **www.miltenyibiotec.com/rea**

Related products

To distinguish true positive events from artifacts in a flow cytometry experiment, it is crucial to include adequate controls. One of the most common types of controls is staining with isotype control antibodies. These antibodies do not recognize specific antigens on target cells, yet mimic all the non-specific binding characteristics of the antibodies used in the experiment.

Thus control antibodies allow users to:

- Confirm specificity of primary antibody binding
- Check for non-specific binding (mainly Fcγ receptors)
- Check for non-specific interactions of fluorochrome

Isotype control antibodies

All REA clones have human IgG1 as their isotype. Therefore, only one type of isotype control, the REA Control antibodies (clone REA293), is needed. These control antibodies are available for surface antigens (REA Control (S) antibodies) and intracellularly expressed antigens (REA Control (I) antibodies).

Isotype controls are mainly used for surface staining. For intracellular stainings, isotype controls should be combined with other negative controls (e.g. staining of unstimulated cells).

A complete list of all REA isotype control antibodies is shown in the isotype control table.

Compensation beads

The MACS® Comp Bead Kit, anti-REA (# 130-104-693) has been developed for optimal compensation of fluorescence spillover of fluorochrome-conjugated REA clones. After staining with fluorochrome-conjugated REA clones, the MACS Comp Beads, anti-REA are used for automated or manual compensation along with the MACS Comp Beads – blank to check all the negative populations.

Isotype control	Order number
Intracellular staining	
REA Control (I)-FITC, human	130-104-611
REA Control (I)-PE, human	130-104-613
REA Control (I)-APC, human	130-104-615
REA Control (I)-PE-Vio770, human	130-104-617
REA Control (I)-APC-Vio770, human	130-104-619
REA Control (I)-PerCP-Vio700, human	130-104-621
REA Control (I)-Biotin, human	130-104-607
REA Control (I)-FITC, mouse and rat	130-104-627
REA Control (I)-PE, mouse and rat	130-104-629
REA Control (I)-APC, mouse and rat	130-104-631
REA Control (I)-PE-Vio770, mouse and rat	130-104-633
REA Control (I)-APC-Vio770, mouse and rat	130-104-635
REA Control (I)-PerCP-Vio700, mouse and rat	130-104-637
REA Control (I)-Biotin, mouse and rat	130-104-623
Surface staining	
REA Control (S)-FITC, human	130-104-610
REA Control (S)-PE, human	130-104-612
REA Control (S)-APC, human	130-104-614
REA Control (S)-VioBlue, human	130-104-609
REA Control (S)-VioGreen, human	130-104-608
REA Control (S)-PE-Vio770, human	130-104-616
REA Control (S)-APC-Vio770, human	130-104-618
REA Control (S)-PerCP-Vio700, human	130-104-620
REA Control (S)-Biotin, human	130-104-606
REA Control (S)-FITC, mouse and rat	130-104-626
REA Control (S)-PE, mouse and rat	130-104-628
REA Control (S)-APC, mouse and rat	130-104-630
REA Control (S)-VioBlue, mouse and rat	130-104-625
REA Control (S)-VioGreen, mouse and rat	130-104-624
REA Control (S)-PE-Vio770, mouse and rat	130-104-632
REA Control (S)-APC-Vio770, mouse and rat	130-104-634
REA Control (S)-PerCP-Vio700, mouse and rat	130-104-637
REA Control (S)-Biotin, mouse and rat	130-104-622

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